Control of *Clostridium perfringens* Spores by Green Tea Leaf Extracts during Cooling of Cooked Ground Beef, Chicken, and Pork[†]

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ABSTRACT

We investigated the inhibition of *Clostridium perfringens* spore germination and outgrowth by two green tea extracts with low (green tea leaf powder [GTL]; 141 mg of total catechins per g of green tea extract) and high (green tea leaf extract [GTE]; 697 mg of total catechins per g of extract) catechin levels during abusive chilling of retail cooked ground beef, chicken, and pork. Green tea extracts were mixed into the thawed beef, chicken, and pork at concentrations of 0.5, 1.0, and 2.0% (wt/ wt), along with a heat-activated (75°C for 20 min) three-strain spore cocktail to obtain a final concentration of \sim 3 log spores per g. Samples (5 g) of the ground beef, chicken, and pork were then vacuum packaged and cooked to 71°C for 1 h in a temperature-controlled water bath. Thereafter, the products were cooled from 54.4 to 7.2°C in 12, 15, 18, or 21 h, resulting in significant increases (P < 0.05) in the germination and outgrowth of *C. perfringens* populations in the ground beef, chicken, and pork control samples without GTL or GTE. Supplementation with 0.5 to 2% levels of GTL did not inhibit *C. perfringens* growth from spores. In contrast, the addition of 0.5 to 2% levels of GTE to beef, chicken, and pork resulted in a concentrationand time-dependent inhibition of *C. perfringens* growth from spores. At a 2% level of GTE, a significant (P < 0.05) inhibition of growth occurred at all chill rates for cooked ground beef, chicken, and pork. These results suggest that widely consumed catechins from green tea can reduce the potential risk of *C. perfringens* spore germination and outgrowth during abusive cooling from 54.4 to 7.2°C in 12, 15, 18, or 21 h of cooling for ground beef, chicken, and pork.

Clostridium perfringens poses a significant risk to the safety of minimally processed meat and poultry products. This ubiquitous organism depends for growth on more than a dozen amino acids and several vitamins (4, 23). Moreover, the organism grows more rapidly than any other foodborne pathogen. The temperature for its growth ranges from 6 to 50°C, with a short doubling time ranging from 7.1 to 10 min, respectively (16). Because of its wide distribution in foods and rapid growth, C. perfringens outgrowth is used to determine if cooling, cooked products remain pathogen free. The current U.S. Department of Agriculture-Food Safety Inspection Service draft compliance guidelines for ready-to-eat meat and poultry products state that such products should be cooled at a rate sufficient to prevent no more than a 1-log increase of C. perfringens cells (32). These federal guidelines also state that cooling from 54.4 to 26.6°C should take no longer than 1.5 h and that cooling from 26.6 to 4.4°C should take no longer than an additional 5 h (32). Additional guidelines allow the cooling of certain cured, cooked meats from 54.4 to 26.7°C in 5 h and from 26.7 to 7.2°C in 10 h.

Changes in consumer attitudes toward the use of synthetic antimicrobial compounds, such as nitrites and sulfites in foods, have stimulated the use of naturally occurring compounds that possess antimicrobial properties. Such biologically active compounds are more likely to be accepted by consumers than synthetic formulations.

Previously, we found that the plant-derived natural compounds carvacrol, cinnamaldehyde, thymol, and oregano oil, as well as the biopolymer chitosan derived from shellfish, individually inhibited *C. perfringens* germination and outgrowth during the exponential cooling of ground beef and turkey (18, 20, 21). The objective of this study was to extend our inhibition studies against *C. perfringens* to naturally occurring and widely consumed green tea catechins.

Green tea, produced from the leves of the plant *Camellia sinensis*, is one of the most popular beverages consumed worldwide. In addition to teas, green tea extracts are sold at retail as health-promoting dietary supplements (nutraceuticals) (24). Numerous studies have been published on the beneficial effects of green tea on human health (3, 13, 28). A major class of biologically active ingredients of green tea is polyphenolic compounds known as catechins. Catechins account for about 10% of the dry weight of teas (3).

A number of published studies describe the antimicrobial effects of tea compounds against a variety of bacteria, bacterial toxins, viruses, and fungi (5, 6, 8, 10, 14, 15, 22,

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1430 JUNEJA ET AL. J. Food Prot., Vol. 70, No. 6

25, 27, 29, 31, 34, 35), which are reviewed in Cushnie and Lamb (7) and Friedman (9). A previous study by Ahn et al. (1) showed that 5 mg of gallocatechin and epigallocatechin gallate isolated from green tea restricted the growth of C. perfringens in vitro. Methyl gallate or gallic acid (10 mg) derived from the Galla-Rhois rhubarb plant also inhibited the spores of C. perfringens (2). However, to our knowledge, the potential of green tea extracts to inhibit the germination and outgrowth of C. perfringens during the cooling of cooked meat and poultry products has not been previously assessed. Accordingly, the main objective of this study was to determine whether green tea extracts with low (green tea leaf powder [GTL]; 141 mg of total of seven catechins per g of green tea extract) or high (green tea leaf extract [GTE]; 697 mg of total of seven catechins per g of extract) catechin levels (12) would inhibit the outgrowth of C. perfringens from spores in retail cooked ground beef, chicken, and pork during chilling.

MATERIALS AND METHODS

Test compounds. Green tea polyphenols (+LKTG6817, lot no. 2395505; GTE) were obtained from LKT Laboratories Inc. (St. Paul, Minn.). GTL (Chromadex BRM 30330-054; ASB-00030330-005) was obtained from Chromadex Inc. (Santa Ana, Calif.). This product is a powdered tea sample with approximately 20% of the polyphenol content of the green tea polyphenols.

GTL and GTE contained the following catechin levels (expressed in milligrams per gram), as determined by high-pressure liquid chromatography (HPLC) (11, 12): [(-)-epigallocatechin, 18.9 and 91.0; (-)-catechin, 4.4 and 23.3; (+)-epicatechin, 7.6 and 89.9; (-)-epigallocatechin-3-gallate, 77.8 and 278.6; (-)-gallocatechin-3-gallate, 4.1 and 27.7; (-)-epicatechin-3-gallate, 23.9 and 175.4; and (-)-catechin-3-gallate, 4.1 and 12.0; their sum, 140.8 and 696.9].

GTL and GTE also contained the following alkaloid levels (expressed in milligrams per gram): caffeine, 43.3 and 18.2; theobromine, 4.2 and 1.5; and theophylline, 0.2 and 2.9; their sum, 47.7 and 22.6.

Test organisms and spore production. *C. perfringens* strains NCTC 8238, NCTC 8239, and ATCC 10288 were obtained from the Microbial Food Safety Research Unit, Eastern Regional Research Unit (Wyndmoor, Pa.), culture collection. An active culture of each strain was freshly prepared in fluid thioglycolate medium, and sporulation was stimulated in Duncan-Strong sporulation medium as previously described (17). After the spore crop of each strain was washed twice and resuspended in sterile distilled water, the spore suspensions were stored at 4°C for up to 6 months. A spore cocktail containing the three strains of *C. perfringens* was prepared immediately prior to use by mixing approximately the same volume of spores from each suspension (~3.0 log spores per g).

Preparation and inoculation of ground meat. Ground beef (93% lean), lean ground chicken, and lean ground pork were obtained from a retail market in Wyndmoor, Pa., and stored frozen (-5°C) until used (approximately 40 days). Green tea polyphenols and GTL were separately mixed into the ground beef, chicken, or pork samples with a KitchenAid mixer to final concentrations of 0.5, 1, and 2% (wt/wt). Duplicate ground beef, chicken, and pork (5-g samples) were then aseptically weighed into low-oxygentransmission Whirl-Pak bags (4 oz [113.4 g] with 120-ml capacity; 3 in. wide by 7¼ in. long; 7.5 by 18.5 cm; barrier film 0.125

ml of oxygen transmission per 100-in. square in 24 h; part no. B01298WA, Nasco, Modesto, Calif.) and inoculated with 1 ml of the heat-shocked C. perfringens spore cocktail to a final concentration of ~ 3.0 log spores per g. The bags were thoroughly mixed manually to ensure an even distribution of the spores in the meat sample. Negative controls consisted of bags containing uninoculated ground beef, chicken, or pork. The bags were then evacuated to a negative pressure of 10^5 Pa and heat sealed with a Multivac gas-packaging machine (model A300/16, Multivac Inc., Kansas City, Mo.).

Cooking and cooling procedures. The Whirl-Pak bags containing the inoculated products were sandwiched between stainless steel wire racks as described previously (30). The product was then completely submerged in a circulating water bath (Exacal, model RTE-221, NESLAB Instruments, Inc., Newington, N.H.). The temperature of the water bath was increased linearly to 71°C within 1 h to mimic the cooking of meat and poultry products in retail food service establishments and the food industry. Next, the bags were removed and chilled immediately in an ice water bath, and the cells were then plated as described below. A second set of racks containing the product, with a C. perfringens spore cocktail of \sim 3.0 log spores per g, for each treatment was cooked (71°C for 1 h) and then transferred to a programmable water bath set at 54.5°C. The bath was allowed to equilibrate at this temperature for 10 min and then chilled at an exponential rate from 54.5 to 7.2°C according to the target chilling times shown in Tables 1 through 3.

Enumeration of bacteria. Immediately after cooking, chilling, or both, samples were removed and enumerated for total germinated *C. perfringens* populations by spiral plating (Spiral Systems Model D Plating Instruments, Spiral Systems, Cincinnati, Ohio) on tryptose-sulfite-cycloserine agar (Difco, Becton Dickinson, Sparks, Md.) as described previously (19). The total *C. perfringens* population was determined after 48 h of incubation at 37°C in a Bactron anaerobic chamber (Bactron IV, Sheldon Laboratories, Cornelius, Oreg.). The lower limit of detection by this procedure is 21 CFU/ml. Uninoculated ground beef, chicken, and pork and cooked ground beef, chicken, and pork without GTE or GTL were used to verify the absence of naturally occurring *C. perfringens*. This verification involved plating on lactose-gelatin and nitrate-motility medium.

Statistical analyses. Two independent trials, in duplicate, as defined by a new batch of ground meat, were performed for each of the exponential chilling rates (12, 15, 18, and 21 h). Data were analyzed with an analysis of variance by the General Linear Models procedure of SAS (release 9.1.3, SAS Institute, Inc., Cary, N.C.). The Bonferroni mean separation test was used to determine significant changes (P < 0.05) before and after chilling in *C. perfringens* populations (log CFU per gram) in treated samples (26).

RESULTS AND DISCUSSION

The programmed time-temperature profiles of the products for the 12-, 15-, 18-, and 21-h chill rates are reported in previous publications (18, 30). These profiles represent extended chilling rates based on the U.S. Department of Agriculture–Food Safety and Inspection Service or the U.S. Food and Drug Administration stabilization requirements for the chilling of uncured, cooked meat and poultry products.

Tables 1 through 3 compare the effects of adding 0.5, 1.0, or 2.0% (wt/wt) of two green tea extracts on the outgrowth and germination of *C. perfringens* in three different meat products: ground beef, ground chicken, and ground

TABLE 1. Control of Clostridium perfringens by green tea leaf extract (GTE)^a and green tea leaf powder (GTL)^b during the cooling of cooked beef^c

Beef sample	12-h chill rate		15-h chill rate		18-h chill rate		21-h chill rate	
	1-h cook	Chill	1-h cook	Chill	1-h cook	Chill	1-h cook	Chill
Control 0.5% GTE	3.34 ± 0.21 3.59 ± 0.07 A	7.24 ± 0.15 6.48 ± 0.17 в	3.17 ± 0.13 3.45 ± 0.27 A	8.04 ± 0.70 6.71 ± 0.45 в	3.24 ± 0.18 3.33 ± 0.20	7.85 ± 0.27 A 6.84 ± 0.41 I	3	
Control 1% GTE				7.89 ± 0.87 4.29 ± 1.94 A	3.24 ± 0.18 3.70 ± 0.23	7.85 ± 0.27 A 6.07 ± 0.70 3	3.15 ± 0.23 3 3.60 \pm 0.30 A	8.02 ± 0.17 A 7.33 ± 0.06 B
Control 2% GTE			2.75 ± 0.60 $3.33 \pm 1.1 \text{ A}$	8.12 ± 0.61 3.69 ± 0.19 A	3.24 ± 0.18 3.79 ± 0.50	7.90 ± 0.27 A 4.26 ± 0.08	3.15 ± 0.23 A 3.79 ± 0.50	7.76 ± 0.14 A 4.40 ± 0.17 A
Control 0.5% GTL	3.27 ± 0.22 3.50 ± 0.24 A	7.24 ± 0.15 7.04 ± 0.02 B	3.27 ± 0.24 3.50 ± 0.24 A	7.62 ± 0.19 7.30 ± 0.17 B				
Control 1% GTL	3.17 ± 0.08 3.57 ± 0.17 A	7.23 ± 0.21 6.97 ± 0.17 B	3.08 ± 0.15 3.22 ± 0.43 A	8.21 ± 0.52 7.71 ± 0.24 B		7.85 ± 0.27 A 7.8 ± 0.54 I	3.15 ± 0.23 3.34 ± 0.32 A	7.76 ± 0.14 $4.7.53 \pm 0.19$ B
Control 2% GTL	3.34 ± 0.21 3.58 ± 0.27 A		3.15 ± 0.23 3.31 ± 0.21 A	8.13 ± 0.61 $7.16 \pm 0.28 \text{ B}$				

^a Previously determined to contain 697 mg of total catechins per g.

pork. Statistical analyses were used to identify treatments that significantly inhibited the pathogen. For each treatment, we compared the means of C. perfringens population densities after the 1-h cooking time and after 12, 15, 18, or 21 h of subsequent exponential chill rates. Those treatments for which the means did not differ significantly (P < 0.05) were considered effective inhibitors of growth of C. perfringens from spores during the cooling of the cooked products from 54.4 to 7.2°C in the 12- to 21-h time frames.

Tables 1 through 3 also show that the chilling of control samples without GTL or GTE, following the 12-, 15-, 18-, or 21-h exponential chill rates, resulted in significant increases (P < 0.05) in the germination and outgrowth of

C. perfringens populations in ground beef, chicken, or pork. The extent of growth obtained in the present study was, in general, similar to the growth rates reported in the literature (18, 20, 21).

GTL did not prevent *C. perfringens* growth from spores in the ground beef, chicken, or pork at the three concentrations tested during the 12-, 15-, 18-, or 21-h exponential chill rates (Tables 1 through 3). This lack of activity may be attributed to the low catechin content of this green tea extract compared with GTE. In contrast, GTE with the higher catechin content was effective at controlling *C. perfringens* population densities following the 12-, 15-, 18-, or 21-h exponential chill rates. Supplementing ground

TABLE 2. Control of Clostridium perfringens by green tea leaf extract $(GTE)^a$ and green tea leaf powder $(GTL)^b$ during the cooling of cooked chicken^c

Chicken sample	12-h chill rate		15-h chill rate		18-h chill rate		21-h chill rate	
	1-h cook	Chill	1-h cook	Chill	1-h cook	Chill	1-h cook	Chill
Control 0.5% GTE	3.46 ± 0.19 3.57 ± 0.19		3.28 ± 0.08 3.54 ± 0.24 A			7.66 ± 0.25 A 6.75 ± 0.13 B		
Control 1% GTE						7.66 ± 0.25 A 5.4 ± 0.38 B		
Control 2% GTE						7.66 ± 0.25 A 4.09 ± 0.23 A		7.66 ± 0.16 A 3.93 ± 0.36 A
Control 0.5% GTL	01.0 - 0.22		3.44 ± 0.22 3.45 ± 0.26 A		i .			
Control 1% GTL	3.30 ± 0.04 3.38 ± 0.03 A					7.66 ± 0.25 A 7.27 ± 0.17 B		
Control 2% GTL	3.46 ± 0.19 3.56 ± 0.12 A		3.33 ± 0.05 3.46 ± 0.13 A	7.30 ± 0.42 6.39 ± 0.20 B	i			

^a Previously determined to contain 697 mg of total catechins per g.

^b Previously determined to contain 141 mg of total catechins per g, about one-fifth of the polyphenol content of GTE.

^c For each treatment and chill rate, two means (1-h cook versus chill) with different letters are significantly (P < 0.05) different, while two means with the same letter are not significantly different. Listed values represent total germinated C. perfringens populations.

^b Previously determined to contain 141 mg of total catechins per g, about one-fifth of the polyphenol content of GTE.

^c For each treatment and chill rate, two means (1-h cook versus chill) with different letters are significantly (P < 0.05) different, while two means with the same letter are not significantly different. Listed values represent total germinated C. perfringens populations.

1432 JUNEJA ET AL. J. Food Prot., Vol. 70, No. 6

TABLE 3. Control of Clostridium perfringens by green tea leaf extract $(GTE)^a$ and green tea leaf powder $(GTL)^b$ during the cooling of cooked pork^c

Pork sample	12-h chill rate		15-h chill rate		18-h chill rate		21-h chill rate	
	1-h cook	Chill	1-h cook	Chill	1-h cook	Chill	1-h cook	Chill
Control 0.5% GTE	3.18 ± 0.15 3.03 ± 0.26 A	7.26 ± 0.09 5.31 ± 0.18 E			U	6.23 ± 1.41 A 6.24 ± 0.73 в		
Control 1% GTE			3.01 ± 0.68 3.36 ± 0.18	5.21 ± 1.10 A 2.66 ± 0.62 A	3.48 ± 0.11 3.58 ± 0.10	6.45 ± 1.69 A 4.02 ± 0.37 A	3.40 ± 0.18 3.37 ± 0.16 A	7.61 ± 0.71 4.92 ± 0.31 в
Control 2% GTE			3.40 ± 0.18 3.64 ± 0.42		3.40 ± 0.18 3.64 ± 0.42	6.22 ± 1.41 A 4.89 ± 0.23 A	3.40 ± 0.18 3.60 ± 0.42 A	7.30 ± 0.34 4.07 ± 0.21 A
Control 0.5% GTL	3.18 ± 0.15 3.42 ± 0.16 A	7.26 ± 0.09 5.92 ± 0.54 B	0.00 - 0.20	7.45 ± 0.54 A 6.23 ± 0.47 B	;			
Control 1% GTL	3.18 ± 0.15 3.20 ± 0.24 A			=		6.17 ± 1.46 а 4.56 ± 0.12 в		7.30 ± 0.34 $7.55 \pm 0.20 \mathrm{B}$
Control 2% GTL		7.26 ± 0.09 5.39 ± 0.10 B	3.03 ± 0.28 3.23 ± 0.17	7.45 ± 0.54 A 5.45 ± 0.39 B	•			

^a Previously determined to contain 697 mg of total catechins per g.

beef with GTE resulted in concentration- and time-dependent inhibition of C. perfringens growth from spores. Specifically, although 0.5% GTE did not inhibit the germination and outgrowth of spores even at the 12-h chill rates, adding 1% GTE to ground beef significantly restricted growth from spores at the 5-h exponential chill rates (Table 1). Note, however, that C. perfringens population densities significantly increased (P < 0.05) from 3.70 to 6.07 log CFU/g following the 18-h chill rate for beef. At a concentration of 2%, GTE completely controlled C. perfringens growth from spores, even during an extended rate and extent of cooling from 54.4 to 7.2°C in 21 h.

With ground chicken, a complete prevention of growth (<0.3 log) occurred with 1% GTE at the 15-h chill rate and with 2% GTE at the 15-, 18-, and 21-h chill rates (Table 2). In general, the inhibition of *C. perfringens* growth from spores in ground pork was similar to that of beef or chicken, except that there was a significant (P < 0.05) inhibition of growth with 1% GTE at the 18-h chill rate (Table 3).

As mentioned earlier, it is well known that polyphenolic compounds in tea exhibit antimicrobial activity. These compounds include catechin, epicatechin, gallocatechin, epigallocatechin gallate, catechin gallate, epicatechin gallate, and gallic acid (8, 10, 31, 33, 34). The distributions of the catechins in green teas and the theaflavins in black teas (collectively known as flavonoids), as well as the biologically active alkaloids caffeine, theobromine, and theophylline in a large number of commercial teas, have been determined (11, 12). In addition, researchers have used HPLC to analyze commercial green tea dietary supplements sold as capsules and powders, including two of the green tea extracts (powders) evaluated in the present study (12). Total catechin levels for the four products sold as powders ranged from 96.0 to 696.9 mg/g of product, a 7.3-fold variation from the highest to lowest value.

Juneja et al. (17) developed a medium for enhancing

the sporulation of *C. perfringens* by supplementing Duncan-Strong sporulation medium with caffeine, theobromine, or theophylline. Thus, the addition of 100 µg of caffeine per ml increased *C. perfringens* spore yields from 4.32 to 6.03 log spores per ml after 24 h of incubation at 37°C. Since these tea alkaloids enhanced sporulation, it is relevant to note, as mentioned earlier, that the green extracts used in the present study contained the following total concentrations of the three alkaloids (expressed in milligrams per gram): GTL, 47.2; and GTE, 22.6. The low activity of the GTL extract may be partly due to the effect of its high content of alkaloids counteracting the inhibiting effects of the catechins on the spores. This aspect merits further study.

The results of the present study show that 1 to 2% levels of a high catechin tea extract containing seven catechins (see "Materials and Methods") were effective at controlling the germination and outgrowth of *C. perfringens* spores in ground red meat and poultry muscle foods. The findings of the present study contrast with the observation by Kim et al. (22), who reported that although tea extracts were active against *Staphylococcus aureus* and *Listeria monocytogenes* in vitro, they were inactive against these organisms in beef. On the basis of the results obtained in the present study, Kim et al. (22) are probably correct in suggesting that the low concentration (0.1%) of tea they used explains the lack of activity in their beef model.

To our knowledge, there are no other published studies on the activities of tea catechins or teas against pathogenic bacteria in meat and poultry products. In a future study, we plan to evaluate the potential of black and white tea extracts that contain biologically active theaflavins against the pathogenic organisms in meat and poultry.

Supplementing raw beef, chicken, or pork with 1 to 2% GTE can reduce the potential risk of *C. perfringens* growth from spores during abusive chilling regimes, thus minimizing the risk to consumers from contaminated food.

^b Previously determined to contain 141 mg of total catechins per g, about one-fifth of the polyphenol content of GTE.

^c For each treatment and chill rate, two means (1-h cook versus chill) with different letters are significantly (P < 0.05) different, while two means with the same letter are not significantly different. Listed values represent total germinated C. perfringens populations.

Our results suggest that the inhibition of growth is related to the catechin content of the two green tea extracts evaluated in this study. The extract with a higher content was much more effective at controlling C. perfringens in the three meat matrices at the 12-, 15-, 18-, or 21-h exponential chill rates than was the extract with the low catechin levels. Use of 1% GTE in beef, chicken, or pork can effectively control C. perfringens growth from spores following 15 h of chilling. C. perfringens populations were controlled after 18 or 21 h of chilling by increasing the levels of GTE to 2% in beef, chicken, or pork under the conditions employed in the present study. The use of tea extracts and of individual tea catechins for the large-scale production of meat and poultry products while guarding against C. perfringens and possibly other sporulating organisms, including Bacillus anthracis, Clostridium difficile, and Clostridium botulinum, as well as the flavor, taste, and appearance aspects of these products, merits further study.

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